

Figure S1, related to Figure 2. BECN1 and UVRAG HDX-MS (A) Difference plot of deuterium incorporation in BECN1 in PI3KC3-C2 versus deuterium incorporation in the presence of Rubicon at 10 seconds, 30 seconds, 60 second, 90 seconds. (B) Sample peptide from PI3KC3-C2 subunit BECN1 protected in the presence of Rubicon. (C) Difference plot of deuterium incorporation in PI3KC3-C2 subunit UVRAG versus deuterium incorporation in the presence of Rubicon at 10 seconds, 30 seconds, 60 second, 90 seconds. (D) Sample peptide from UVRAG protected in the presence of Rubicon.

A

PIKBD (unbound)



B

PIKBD (C2-bound)

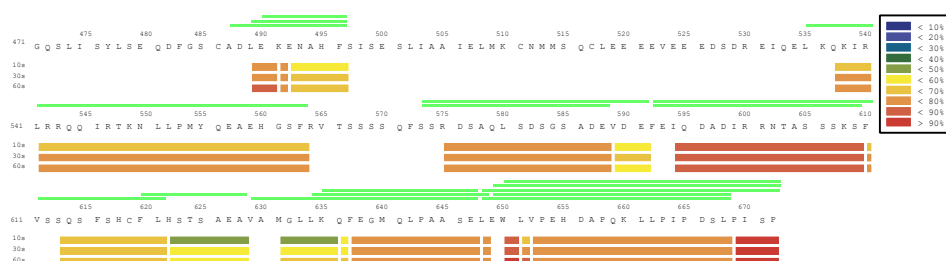


Figure S2, related to Figure 2. HDX-MS exchange patterns on Rubicon PIKBD. (A and B) Heat map showing hydrogen deuterium exchange pattern on PIKBD apo (A) and C2 bound state (B). Peptides are represented using rectangular strips above the protein sequence. Absolute deuterium uptake after 10 seconds, 30 seconds, and 60 seconds is indicated by a color gradient below the protein sequence.

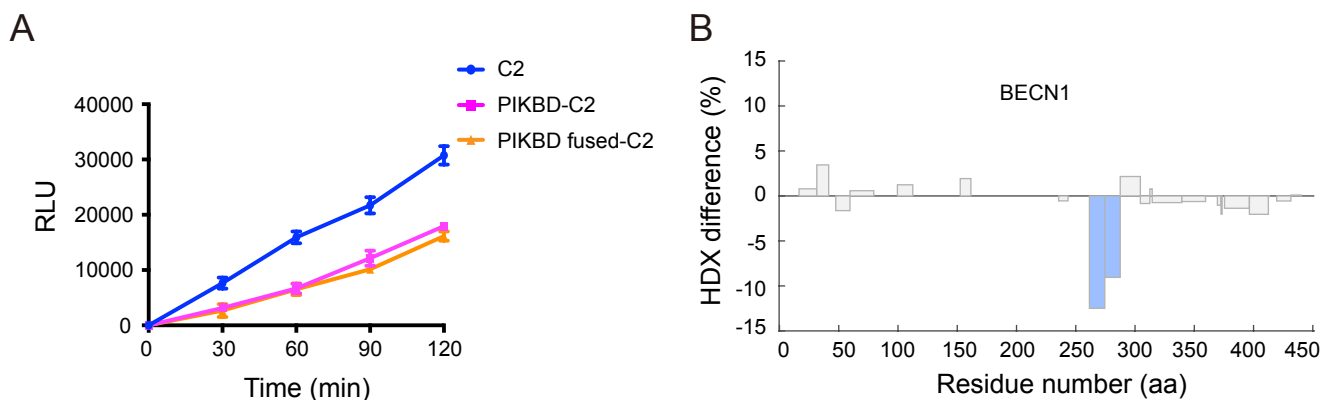
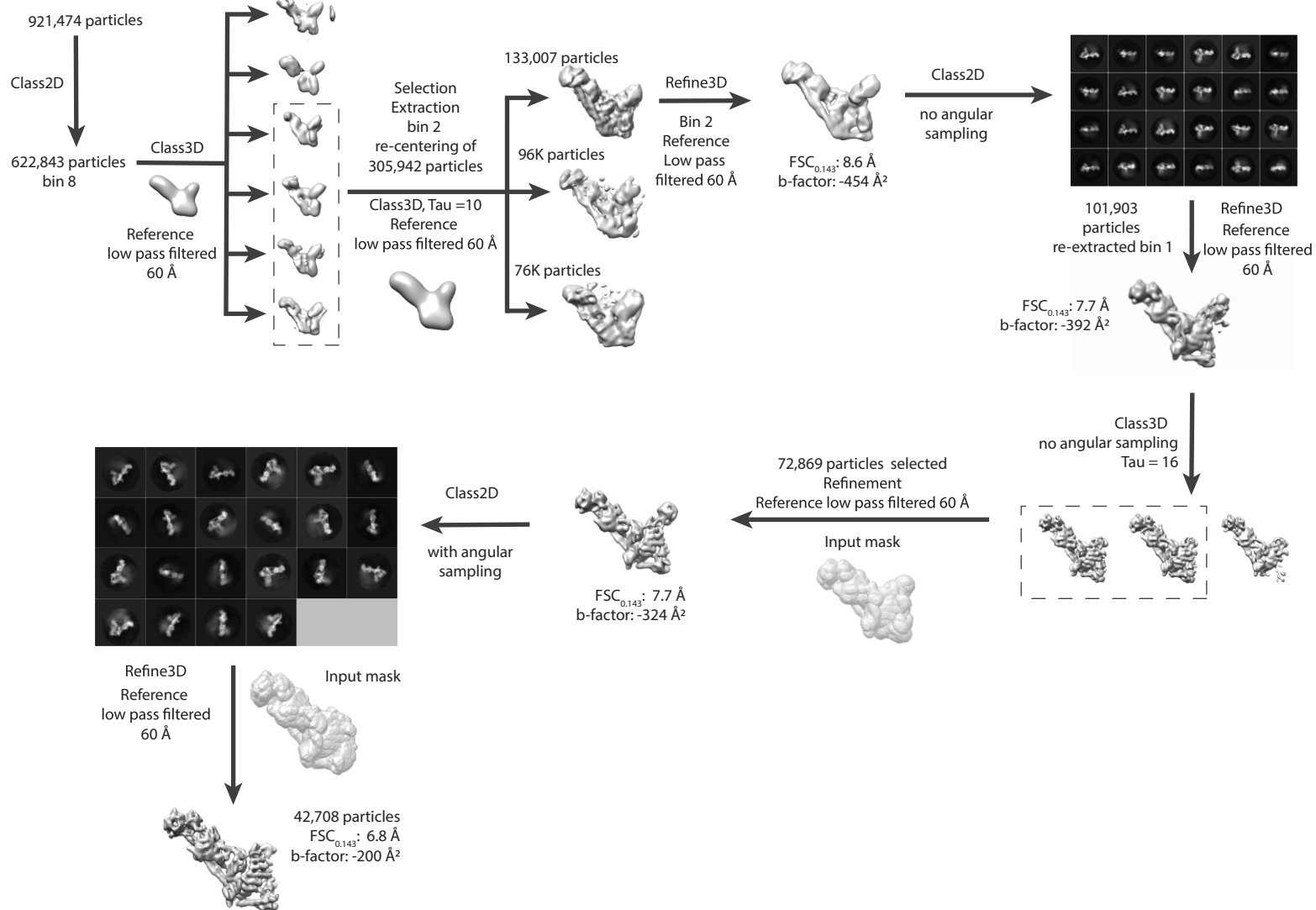


Figure S3, related to Figure 4. Activity and HDX protection of Rubicon BECN1 fused-C2. (A) Activity assays of wild-type PI3KC3-C2 (blue), versus Rubicon PIKBD-C2 unfused (pink) and fused to BECN1 (orange), complexes were incubated with SUVs for indicated time, and ATP consumption was measured. RLU, relative light units. (B) Difference plot of deuterium incorporation in BECN1 in PI3KC3-C2 versus deuterium incorporation in the presence of Rubicon at 30 seconds.

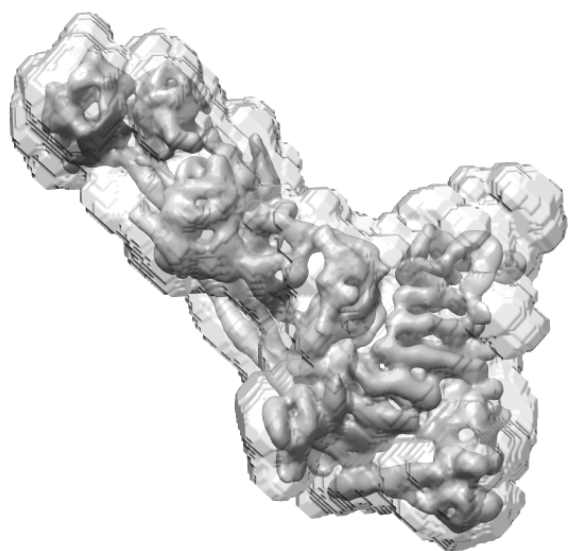
A

PI3KC3-C2: Rubicon PIKBD

Whole data set



B



C

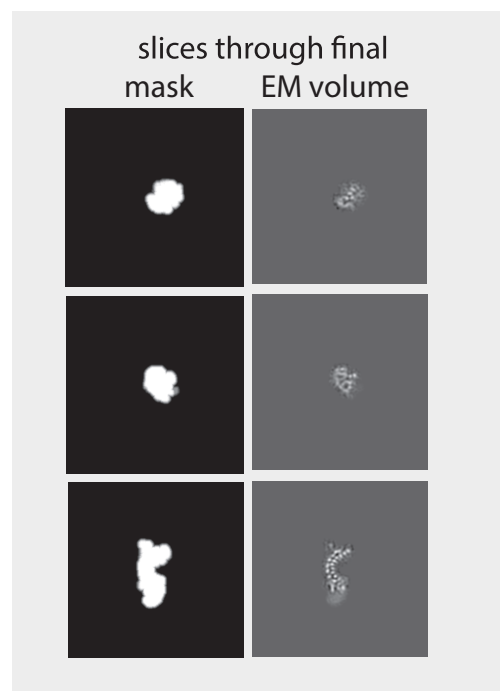


Figure S4, related to Figure 4. Classification workflow. (A) CryoEM data on PI3KC3-C2: Rubicon PIKBD was processed starting with 921,474 particles, subjected to 2D yielding 622,843 particles that went through 3D classification resulting in 305,942 particles. Re-grouping of 305,942 particles and another round of 3D classification yielded a class of 133,007 particles, which were refined. Another round of 2D classification removed more junk classes, leaving 101,903 particles, which were further refined. Another round of 3D classification removed broken particles, leaving 72,869 particles. These particles were refined, and then subjected to 2D classification with angular sampling, leaving a set of 42,708 very clean particles that refined to 6.8 Å and a bfactor of -50 Å². (B) Sample masking procedure for refining PI3KC3-C2:Rubicon-PIKBD. This mask was generated using the map from the refinement, set at sigma level 6 (initial binarisation threshold 0.0073), the map was then extended 5 pixels (5.75 Å) and a soft-edge was added of 8 pixels (9.2 Å). (C) Slices through the mask and the final post-processed map confirm that the mask is soft, and that the features of the map can be visualized.

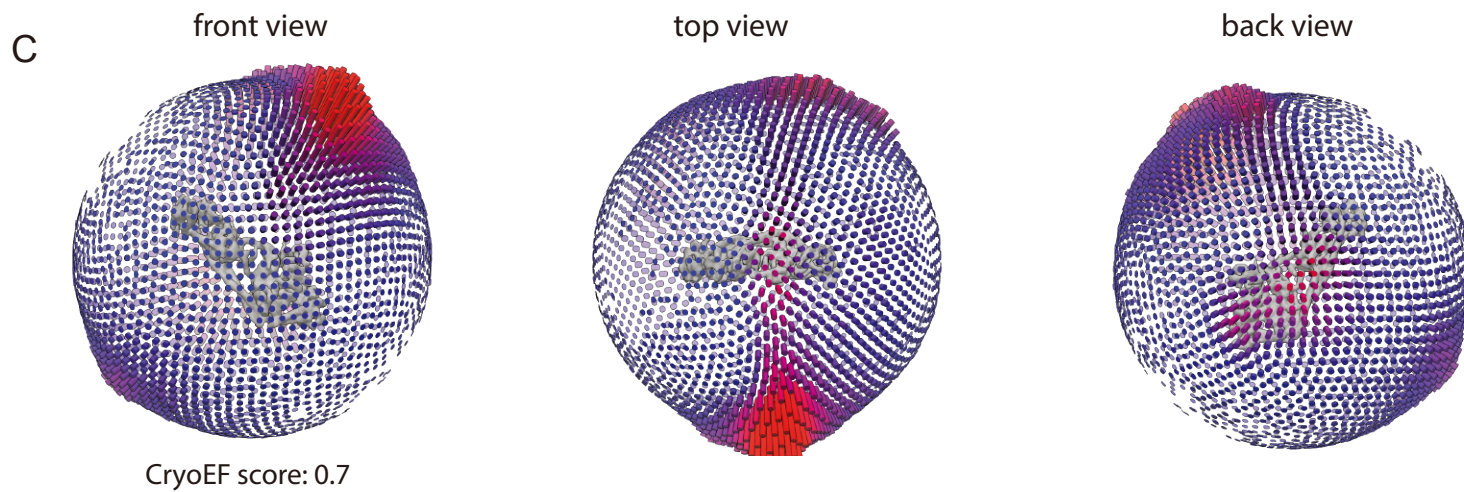
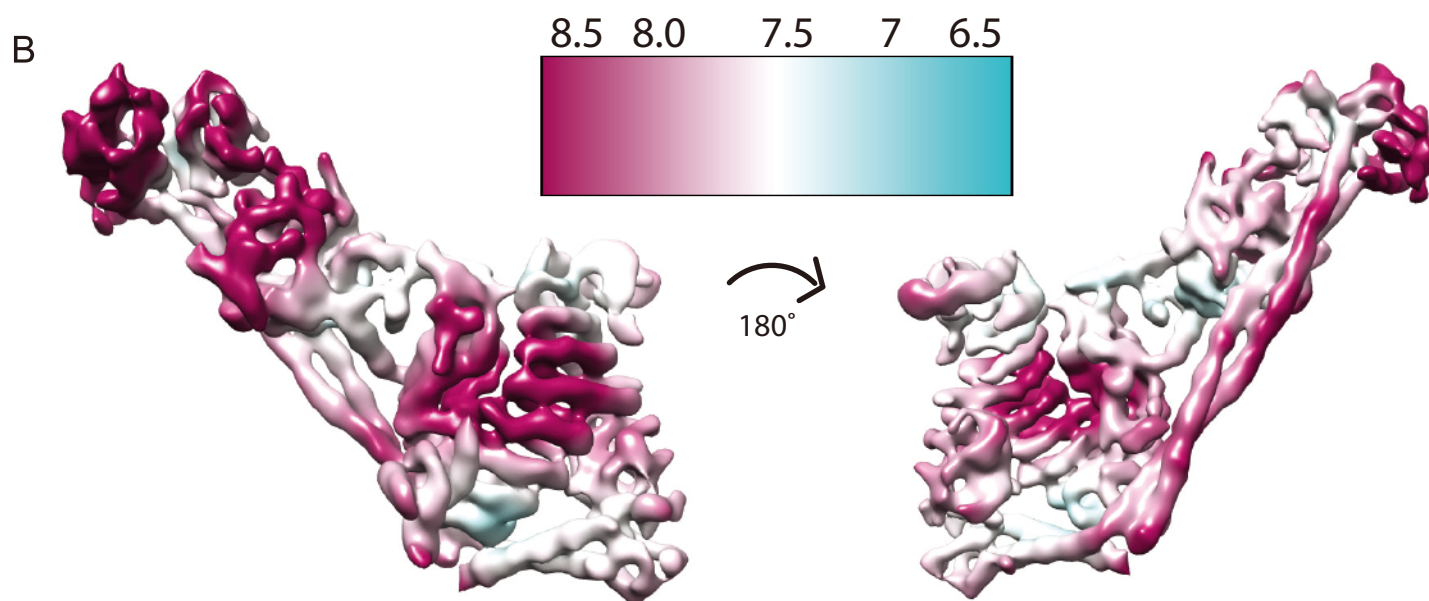
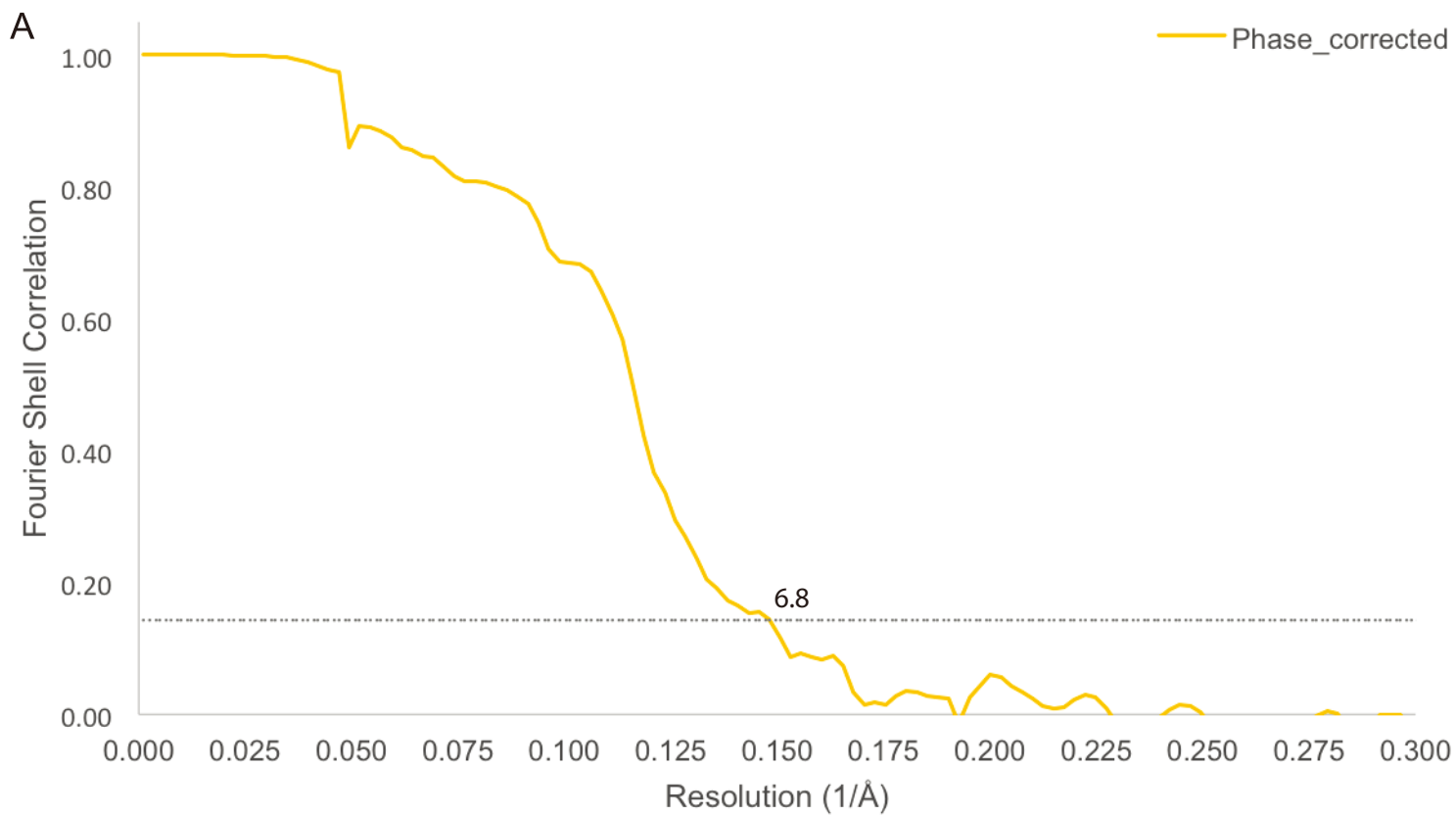


Figure S5, related to Figure 4. Representative cryo-EM data and metrics. (A) Whole reconstruction gold-standard FSC resolution estimation of PI3KC3-C2:Rubicon-PIKBD. (B) Local resolution estimation of the final cryoEM map. (C) Angular distribution of the final refinement, and a cryoEF score of 0.7.

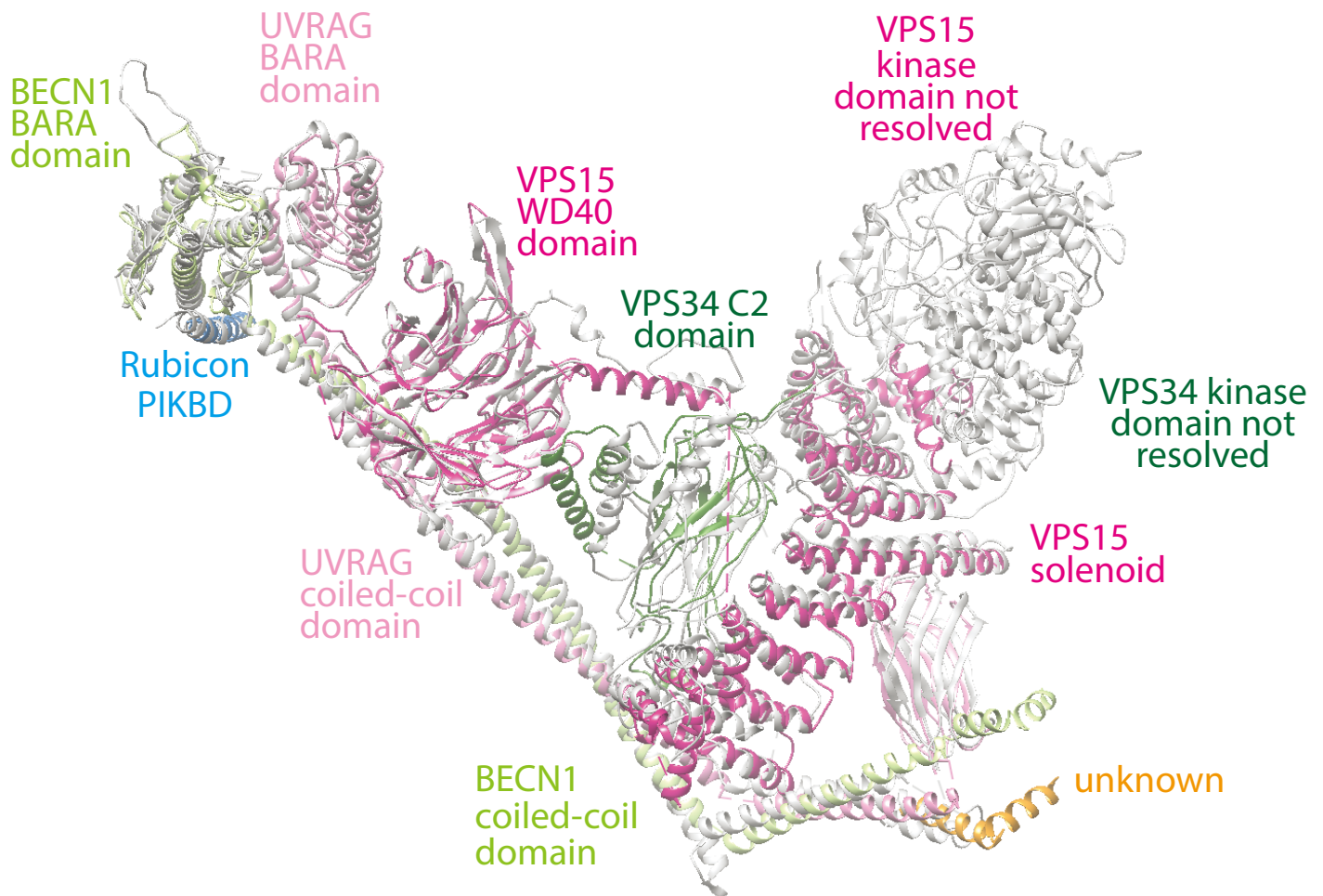


Figure S6, related to Figure 4. Comparison of human cryo-EM and yeast crystal structures of PI3KC3-C2. An overlay of the yeast model 5DFZ (white) and the model generated in this study. The models were aligned through the C2 domain of VPS34.

Table S1, related to Figure 4. Statistics of cryo-EM data collection and processing.

Map	PI3KC3-C2:Rubicon PIKBD
Data collection	
Microscope	Krios
Voltage (kV)	300
Detector	K2 Summit
Recording mode	Super resolution
Magnification	105,000x
Movie micrograph pixel size (Å)	1.15
Dose rate (e-/Å ² /sec)	9.75 seconds
Number of frames per movie micrograph	39
Frame exposure time (ms)	250
Movie exposure time (ms)	9500
Total dose (e-/Å ²)	59.7 e-/Å ²
Defocus range (um)	-0.75 to -2.00
EM data processing	
Number of movie micrographs	4,202
Box size	352
Particle number (total)	921,474
Particle number (post 2D)	622,843
Particle number (post 3D)	133,007
Particle number (used in final map)	42,708
Refinement angular accuracy (°)	3.08 degrees
Refinement offset accuracy (Å)	1.464 pixels
Symmetry	C1
Map resolution (FSC 0.143)	6.8 Å
Map sharpening B-factor (Å ²)	-200
CryoEF score	0.7
CryoEF worst/best PSF res (Å)	4.90/2.29